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Etiological Studies on the Banana Bunchy Top[1]

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Abstract: Hand-section of tissues of bunchy top diseased banana plants were examined under a reflecting fluorescence microscope. A distinctly specific bright fluorescence was observed in these phloem tissues of lamina veins, midribs, sheaths, corms and roots. Symptomless suckers taken from infected stool also gave a weak fluorescent reaction. These specific reactions were phloem-limited, and were not observed in the healthy banana plants. Leaf sections prepared from both diseased and healthy plants of banana were also examined under light microscope after treatment with Dienes' stain. No specific blue staining was noticed in these phloem tissues. These results indicated that the banana bunchy top disease is probably not of a mycoplasmal etiology and that a phloem-limited etiology may possibly be involved.

Banana bunchy top (Bbt) was first reported as a new disease destructive to banana cultivation in Fiji Island in 1891 by Knowless and Jepson (cited by Sun, 1961)[4]. The disease was considered to be of viral origin on the basis of its transmission by the banana aphid *Pentalonia nigronervosa* Coq.[4,5]. Chen (1972) made electron microscopy of Bbt infected banana plants and observed mycoplasma-like organisms (MLOs) in the epidermal cells in the disease material[1]. This observation led him to propose a mycoplasmal etiology for banana bunchy top, in spite of the fact that epidermal cells do not appear to be a normal site for MLO localization. In either case, the evidence seems inconclusive and a confirmation is needed.

Recently, some simple techniques for diagnosing plant diseases due to phloem-limited viruses or mycoplasmas by light microscopy have been reported[2,3]. Namba *et al.* (1981) used a reflecting fluorescence microscope (RFM) for the detection of plant infections by phloem-limited viruses and MLOs[3]. Deeley *et al.* (1979) described a method using Dienes' stain (DS) for the detection of plant MLOs[2]. We made attempts to clarify the etiological problem of banana bunchy top by simultaneous applications of the RFM and DS techniques. The distribution and relative intensity of fluorescent reactions in the phloem tissues of Bbt diseased plants were also studied.

Bunchy top diseased banana plants showing typical symptoms (Fig. 1a), symptoml-

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ess suckers from diseased plants and healthy plants of Hsien-jen chiao (*Musa sapientum* L.) were collected from banana plantations in the Taichung and Nantou areas. Hand-sections of plant tissues from leaves, sheaths, corms and roots were examined under a reflecting fluorescence microscope (RFM, Zeiss Standard XBO-75W) after the method of Namba *et al.* (1981) [3]. Hand-sections of phloem tissues from both diseased and healthy banana plants were also examined for Dienes' stain reactions under a light microscope (LM) [2].

Direct fluorescence microscopy of tissue sections from 21 Bbt diseased banana plants showed a distinctly bright yellow or greenish yellow color in the phloem cells, a yellow, yellowish green or green color in the vessel well, a pale green or yellow color in the cortex and a red or reddish brown color in the chloroplasts under different filter combinations. The specific bright yellow fluorescence was observed in the cytoplasm of phloem cells only (Fig. 1b, c, e). No such fluorescence was observed in the phloem tissues of 162 tested healthy plants (Fig. 1e). Accordingly, the observed phloem reaction was specific to the infection of banana plants with bunchy top disease. The specific yellow fluorescence was most intense in sections prepared from midribs. Moderate amount of fluorescence was observed in sections of sheaths and corms. The roots and symptomless suckers from diseased plants gave a rather weak fluorescent reaction (Table 1). This result indicates that a phloem-limited etiology may possibly be involved [3].

Table 1. Distribution of fluorescent reactions specific to banana bunchy top infection in different parts of banana plant observed under reflecting fluorescence microscope

Materials for examination	No. of plants tested	Relative intensity of fluorescence in phloem tissues(a)
Diseased plant	21	
Leaf		
Lamina veins		+++
Midribs		++++
Sheath		
Upper parts		+++
Middle parts		++
Basal parts		++
Corm		
Central parts		++
Outer parts		++
Root		
Small roots		±
Branched roots		±
Main roots		+

Symptomless sucker	4	
Veins		+
Sheaths		+
Healthy plant	162	
Leaf		
Lamina veins		−
Midribs		−

(a)Number of plus symbols indicate severity of fluorescent reaction in phloem cells.
++++ : most severe. +++ : severe. ++ : intermediate.
+ : mild. ± : very mild. − : none.

Light microscopic observations of both diseased and healthy tissues after treatment with Dienes' stain did not reveal a specific blue staining in the phloem, though some materials of unknown nature were observed to accumulate in the infected phloem cells (Fig. 1d, f). This result indicates that the Bbt may not have a mycoplasmal etiology [2].

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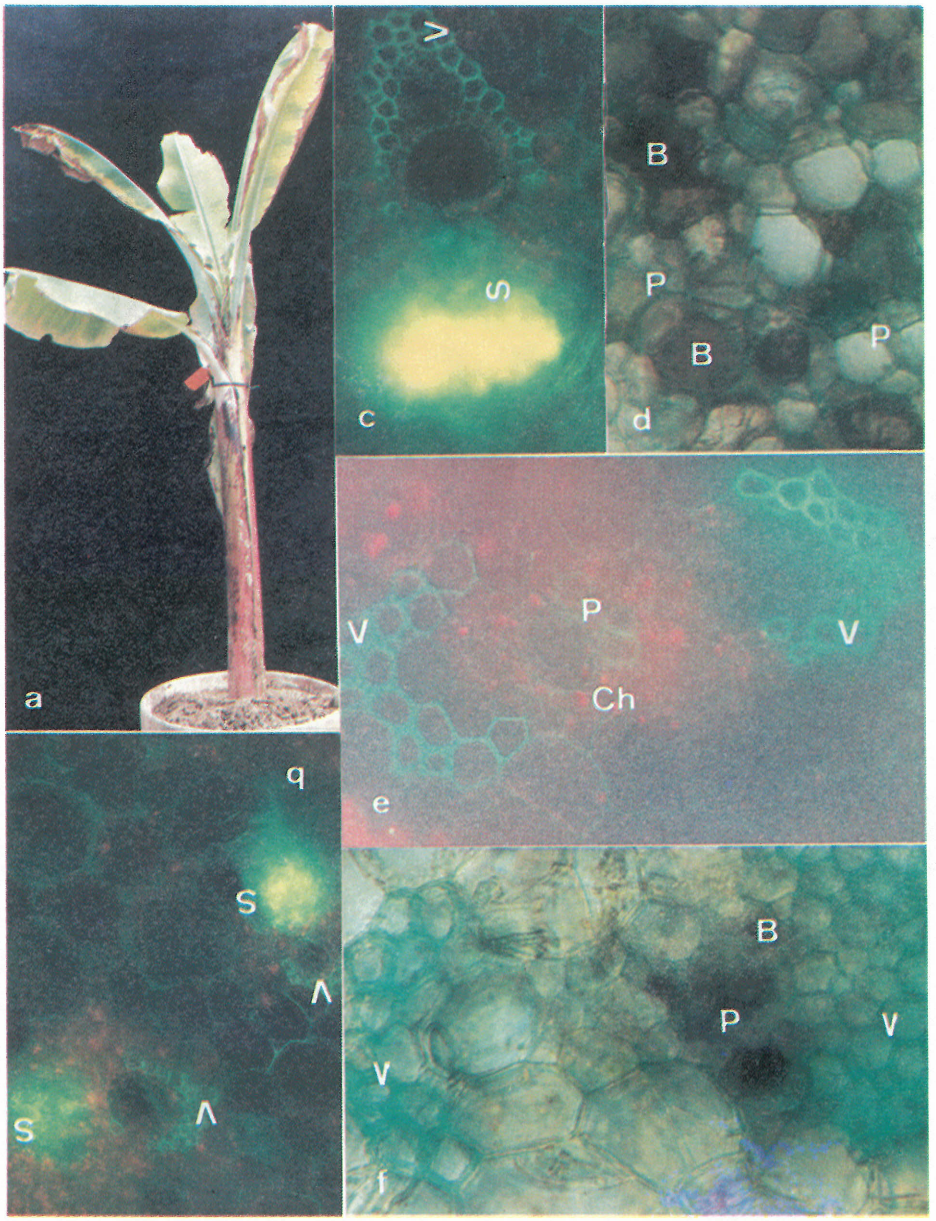

Fig. 1. Fluorescence and light microphotographs of vascular bundles of banana plants infected with bunchy top. Specific fluorescence reaction was observed only in the phloem cells of diseased plants and none of the infected phloem cells stains blue with Dienes' stain. S: specific fluorescence, P: phloem cells, V: vessel cells, Ch: chloroplasts, B: brown color in infected phloem cell. (a) A bunchy top (Bbt) diseased banana plant. (b) Midrib of Bbt plant. (c) Specific bright yellowish fluorescence in infected phloem cells. (d) Brown color in infected phloem cells treated with Dienes' stain under LM. (e) Lamina vein of healthy banana plant. (f) Midrib of Bbt plant treated with Dienes' stain.

香蕉萎縮病病原之研究[1]

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摘 要

香蕉萎縮病病株組織之徒手切片，經反射型螢光顯微鏡觀察結果，在葉片、主脈、葉鞘、球莖、根部及病株之無病徵吸芽的篩管細胞，呈現顯著特殊螢光反應。這些特殊螢光反應局限於篩管組織。健全蕉株之篩管組織則無此反應。同時香蕉病株與健株葉部切片，亦以 Dienes' stain 染色處理後，經光學顯微鏡觀察，結果在該篩管組織皆無特殊性藍色染色反應，此項結果表示本病病原並非擬菌質體。本病病原或其與寄主植物反應生成物分佈於香蕉病株之葉部、葉鞘、球莖、根部及病株之無病徵吸芽苗之篩管組織。

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